DNMT1 and Cancer: An Electrifying Link

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Aberrant epigenetic methylation is linked to the onset and progression of cancer. In this issue of *Chemistry & Biology*, Furst and Barton (2015) describe a sensitive electrochemical assay that can detect hyperactive epigenetic methylation in tumor tissue.

Since the initial provocative evidence that DNA acts as a wire to facilitate electron transfer between two redox-active intercalators, Jacqueline Barton and her coworkers have delineated the fundamental features of DNA-mediated charge transport and illustrated how these features may be used by nature (Genereux and Barton, 2010; Grodick et al., 2015). In addition, the Barton laboratory has developed many practical applications that exploit the "wire-like" behavior of DNA (Boon et al., 2003). In this issue, Furst and Barton (2015) show that a DNA charge transport based sensor may be used as a new diagnostic tool for identifying aberrant epigenetic markers associated with carcinogenic transformation.

Epigenetics encompasses a range of cellular mechanisms that modulate transcription at the level of individual genes. Analogously to adjusting the bass or treble frequencies on a stereo, epigenetic processes can facilitate biological complexity by allowing one gene to be muted or silenced while another may be amplified. These mechanisms, such as DNA methylation and histone modification, do not alter the primary sequence of the DNA bases, but produce potentially heritable changes to the phenotypic expression of the genome. The epigenetic addition of methyl groups to the 5-position of cytosine bases in a DNA sequence results in a gradual silencing of the target gene. Both hypomethylation and hypermethylation of oncogenes and of tumor suppressor genes, respectively, may lead to cancer. Maintenance of the correct methylation patterns in the genome is crucial as evidenced by the link between anomalous methylation events and early stages of cancer cell development (Baylin and Herman, 2000).

Currently used methods for detecting abnormal methylation activity suffer from a number of flaws that make them unsuitable for clinical diagnostics. Many groups have attempted to use the expression levels of the predominant human DNA methylating enzyme DNMT1 as a marker of methylation status; however, these measurements have not typically correlated well with cellular methylation ability because total protein does not account for inherent enzyme activity. The most sensitive and reliable method directly tracks the activity of DNMT1, a difficult and time-consuming process that uses radiolabeled *S*-adenosylmethionine as the methyl donor. Alternatively, methylation sites in the genome can be mapped as a proxy measure of methylation activity in a cell. One such method uses DNA digestion by methylation-sensitive restriction endonucleases, followed by DNA detection; however, this analysis is blind to regions of the genome that do not have restriction sites. Bisulfite sequencing is unaffected by this bias but is typically cost-limited in scope and therefore ill-suited to analyze the global genomic methylation behavior of cells (Fraga and Esteller, 2002). Notably, methods that map methylation sites in genomic DNA produce only a single, static picture of cellular DNA methylation and may not reflect early upticks in methylation activity.

In this issue, Furst and Barton (2015) demonstrate a simple and sensitive method for monitoring aberrant DNMT1 methylation activity in tissue samples (Muren and Barton, 2013; Furst et al., 2014a). Key features of the assay are the use of a two-electrode system coupled with a catalytic redox cycle that amplifies the electrochemical signals. In this system, DNA-mediated charge transport is detected by reduction of the redox intercalator methylene blue (MB) to leucomethylene blue (LB); LB in turn reduces ferricyanide to ferrocyanide, reoxidizing itself back to MB. The DNA-mediated signals are detected at the second electrode by current produced upon re-oxidation of ferrocyanide (Furst et al., 2014b). The duplex DNA acts as an antenna that transmits the electric signal between the electrodes, and therefore the amount of signal generated is directly related to the amount of intact duplex DNA. Digestion of the immobilized DNA "antennae" by a restriction enzyme short circuits the device and results in loss of signal (Figure 1A). DNA methylation by DNMT1 provides protection of the duplex DNA from being cut by the restriction enzyme, and therefore the sustained electrochemical signal directly reports on DNMT1 methylation activity (Figure 1B).

This modular multiplexed electrode system reveals distinctly increased DNMT1 methylation activity in cell extracts from tumor cells relative to that of tumor-adjacent normal cells. Because this altered activity precedes the resulting changes in methylation patterns on genomic DNA, this method holds much promise for early detection of precancerous transformations. Moreover, this strategy may be adapted to create versions of this platform that report on the activity of a variety of DNA binding proteins and enzymes implicated in epigenetics and cancer. As one potential example, the approach used here for DNMT1 activity could be modified to detect faulty removal of aberrant DNA bases by base excision repair glycosylases that have been linked to mutagenesis and carcinogenesis (David et al., 2007). Indeed, we can envision diagnostic arrays of such electrode assemblies where each is tailored to respond to a specific oncogenic activity such as hypermethylation or aberrant DNA repair, allowing clinicians to visualize initial pre-malignant stages of

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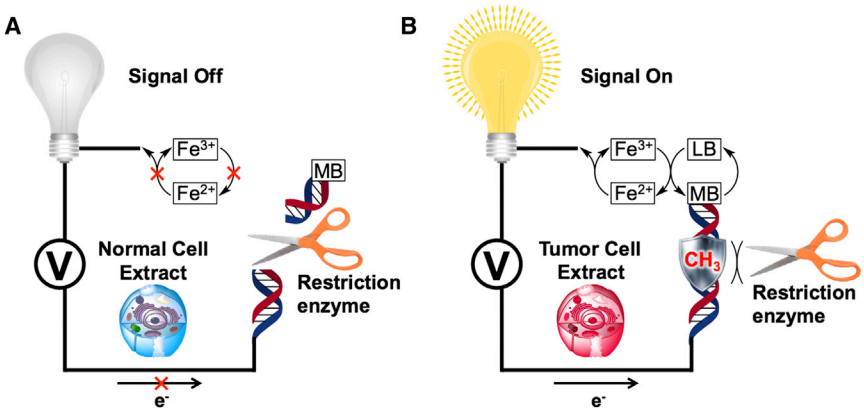

Figure 1. DNA Charge Transport Based Sensor for Measuring Levels of DNA Methylation
(A) The DNA "wire" is not methylated in the presence of normal cell extracts, leaving it susceptible to cleavage by the restriction enzyme (*BssH*II), which eliminates the electrochemical current.
(B) DNA methylation by tumor cell extracts protects the DNA "wire" and maintains the electrochemical signal (represented by the lit bulb).

carcinogenesis and providing more effective opportunities for early intervention. In summary, Furst and Barton demonstrate a robust yet sensitive electrochemical assay that surpasses currently applied diagnostic methods and that strongly correlates enzymatic activity of DNMT1 to tumorigenesis.

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